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Eco-biological Approaches for Nitrogen Removal: The Role of Macrophytes and Microbial Communities in Sustainable Wastewater Treatment

Belkacem HAMMADI [1*], Abdellali FEKIH [3], Naoual ZOBEIDI [2] , Khaled MANSOURI [2], Abdellatif RAHMANI [2], Noureddine GHERRAF [4], Ahmed TABCHOUCHE [2*]

[1] Department of Environmental Chemistry, Laboratory of Water and Environmental Engineering in Saharan Environments, Kasdi Merbah University, Ouargla, Algeria

[2]University of Ouargla, Faculty of Applied Sciences, department of process engineering, System Dynamics, Interaction and Reactivities Laboratory, Ouargla, Algeria.

[3] Department of Physics, Laboratory of New and Renewable Energies in Arid Zones (LENREZA), Kasdi Merbah University Ouargla, Algeria.

[4]Laboratory of Natural Resources and Management of Sensitive Environments, university of Oum El Bouaghi, Algeria.

**Corresponding authors E-mail:* hammadinacer64@gmail.com ; tabchouche.ahmed@univ-ouargla.dz

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Abstract

This year-long study (2024) assessed the efficiency of *Juncus effusus* in treating domestic wastewater in Biskra, southern Algeria. The region is characterized by hot, dry summers and cold winters. A hybrid vertical–horizontal flow system was designed for the experiment. It consisted of three planted basins and three unplanted basins as controls. The model was installed near the conventional wastewater treatment plant of Biskra. Each basin had a capacity of 150 L with a 10 cm sand layer and a 50 cm gravel layer. Young stems of *Juncus. effusus* were planted at a density of 40 roots/m². Irrigation was applied twice per month with 120 L of pretreated wastewater. The wastewater was retained for 15 days before collection from the outlets of the barrels. Laboratory analyses were conducted on both influent and effluent samples. The results revealed significant nitrogen removal efficiency across pollutants. Ammonium (NH_4^+) removal averaged 74.62% during the study. Nitrate (NO_3^-) removal reached 86.96%, whereas nitrite (NO_2^-) removal reached 74.71%. The planted basins consistently outperformed the unplanted controls. The system demonstrates eco-sustainable potential for water management and irrigation in arid regions

Keywords: Biskra, *Juncus effusus*, ammonium, nitrate, nitrite, treatment and purification **.**

1. Introduction

Vascular aquatic plants, algae, and macroalgae with well-developed tissues play fundamental roles in the structure and functioning of wetlands and marshlands. As primary producers, most of these organisms are flowering phototrophic plants (angiosperms), which assimilate inorganic carbon via solar energy to generate organic matter that supports animals, bacteria, and fungi [1] [2] [3] [4]**;** Cronk and Fennessy **2001**). The presence of hydrophytes is therefore a key determinant in identifying wetland ecosystems, where they contribute significantly to physical, microbial, and biochemical processes, including the treatment of nitrogen pollution, one of the most hazardous contaminants affecting humans, animals, plants, and birds. Nitrogen, which constitutes approximately 78% of the biosphere, represents a paradox in the environmental sciences. On the one hand, it is indispensable for the growth of living organisms and agricultural productivity; on the other hand, its excessive release into the environment disrupts the ecosystem balance, causes eutrophication, biodiversity loss, and soil acidification, and contributes to greenhouse gas emissions such as N_2O. Its complex biogeochemical cycle and the high toxicity of certain forms, notably ammonia (NH_3), make it a priority pollutant in many countries [5][6]. In Canada, wastewater treatment plants discharge approximately 62,000 tons annually of ammonium (NH_4^+), exerting major ecological impacts, whereas in wild animals such as pigs, nitrogen excretion reaches 13–15 g/day, largely in the form of ammonia, urea, and uric acid. In Algeria, reliable data on nitrogen pollution remain scarce. Despite the existence of nearly 950 wastewater treatment plants, most rely on mechanical methods and discharge insufficiently treated effluents directly into natural ecosystems. Given that nitrogen is both a vital nutrient and a serious pollutant, evaluating the capacity of plants and their associated microbial communities to remove this compound is of considerable ecological and practical importance. This research, which was conducted over the course of 2024, investigated the cooperative interactions between macrophytes and microorganisms in terms of nitrogen removal within aquatic systems. The main objective of this study was to assess the efficiency of these biological processes as alternative approaches for wastewater treatment. A further goal is to explore the potential generalizability of this method in rural agricultural areas lacking sewage networks and mechanical treatment facilities, with the broader aim of promoting sustainable, nature-based solutions for wastewater management.

2. Materials and methods

2.1.Presentation of the Biskra Region

Biskra Governorate, located in the southeastern part of the People’s Democratic Republic of Algeria and covering 22,375.95 km², is administratively divided into 12 departments and 33 municipalities. It lies approximately 450 km from the capital, Algiers, and is bordered by Batna to the north, Khenchela to the northeast, M’sila to the northwest, Djelfa to the southwest, Oued to the southeast, and Ouargla to the south [7] (**Fig. 1**). The region is internationally renowned for its agricultural potential, particularly the production of “Deglet Nour” dates, which are considered one of the finest cultivars worldwide. Extensive greenhouse farming ensures year-round fruit and vegetable production, consolidating Biskra’s role as a strategic agro-industrial hub. Livestock breeding and agri-food processing further increase its contribution to local and national economic development. Commercially, it serves as a dynamic distribution center for agricultural products, reinforcing its pivotal role in national food security. Climatically, Biskra is characterized by a semiarid to arid environment, with extremely hot, dry summers, high solar radiation, and occasional strong winds. Low and irregular precipitation renders agriculture highly dependent on irrigation systems and subterranean water resources.

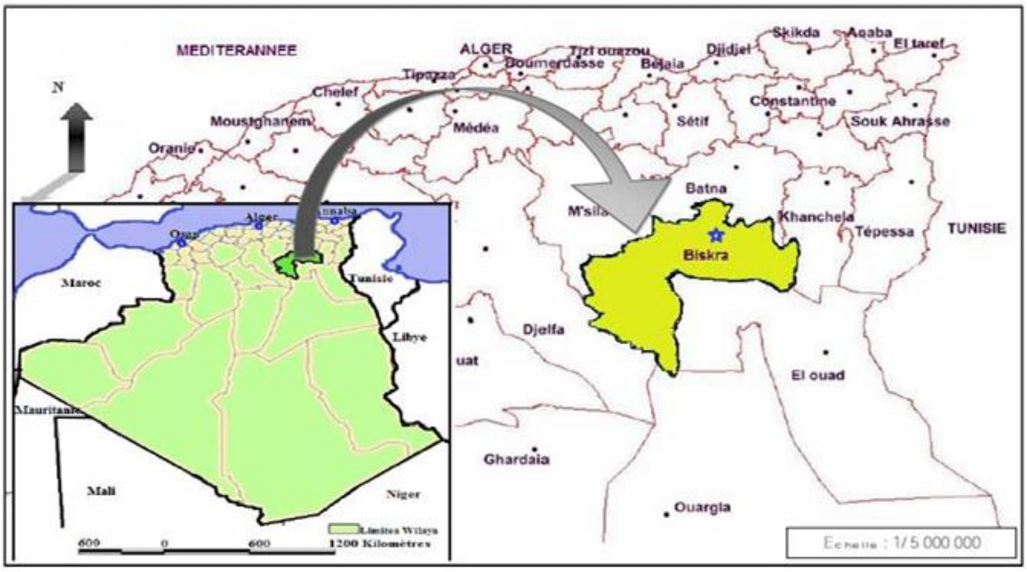

Fig 1. Situation of Biskra on the map of Algeria [7].

2.2.Description of the plant *Juncus effusus* used in the experiment

Juncus effusus L. is a perennial herbaceous plant belonging to the Juncaceae family. It possesses an extensive fibrous root system that morphologically resembles that of grasses. The stems are numerous, arising from a thin, cylindrical, and erect base, and the plant often exhibits a pinkish hue owing to its herbaceous nature. Vegetative propagation occurs through underground buds, which are arranged in a circular manner and give rise to sterile flowering shoots. Under favorable conditions, particularly in humid and cold climates such as those of England and western Ireland, the plant attains a maximum height of approximately 1.5 m. The leaves are reddish-brown, whereas the rhizomatous stems grow horizontally at a depth of 1.5–3 cm below the soil surface, forming dense mats. Sexual reproduction occurs during the summer season, with flowering observed mainly in July and August [8]. The annual rhizome elongation rate is estimated at approximately 2 cm. Some rhizomes also grow vertically, reaching depths between 15 and 22.5 cm. Observations conducted in Oxford, USA, reported that overwintering buds of *J. effusus* exhibit physiological adaptations that ensure survival at low temperatures. Morphological changes are especially evident in the buds, with the lower parts remaining green and the upper regions displaying a reddish coloration [9][10]. Fig. 2 illustrates the plant at full maturity, while Table 1 summarizes its taxonomic classification.

Fig 2. Represents the growth of plants around wetlands.

Table 1: Scientific Classification of Plants

Rank	Name
Scientific Name	*Cyperus papyrus*
Domain (Scope)	Eukaryota
Kingdom	Plantae
Division/Phylum	Magnoliophyta (Angiosperms)
Class	Liliopsida (Monocotyledons)
Order	Poales
Family	Cyperaceae
Genus	*Cyperus*
Type	Herbaceous, perennial, aquatic plant

Table 1 presents the scientific classification and morphological specifications of the plant employed in the treatment of domestic wastewater in Biskra, Algeria.

2.3 Description of the experimental station

The experimental station was established adjacent to the conventional wastewater treatment plant of the city of Biskra at the end of 2023, specifically in December, just before the beginning of 2024, which marked the start of the experimental work. The aim was to obtain treated wastewater from a conventional station in Biskra. The Experimental Station consists of two installations that are identical in terms of area, size, irrigation method, and materials used in their construction. Figure 3 illustrates the installation method. Each installation is composed of three basins arranged as parallel rectangles, wider at the top and slightly narrower at the bottom, interconnected by a plastic tube equipped with a tap in the middle to regulate both the pressure of the water flow and the hydraulic retention time. The retention time in the first and second basins is set at 10 days, whereas that in the third basin is 5 days, resulting in a total experimental retention period of 15 days. This configuration allows for two experimental cycles per month. The basins in both installations, one serving as a control (uncultivated) and the other planted with *Juncus effusus* at a density of 40 roots/m^2, are filled from bottom to top with 50 cm of gravel (30/20 mm) with 10 cm of sand. The capacity of each basin in both installations is 150 L. These parameters are consistent with those reported in previous studies [11][12] (Fig 3).

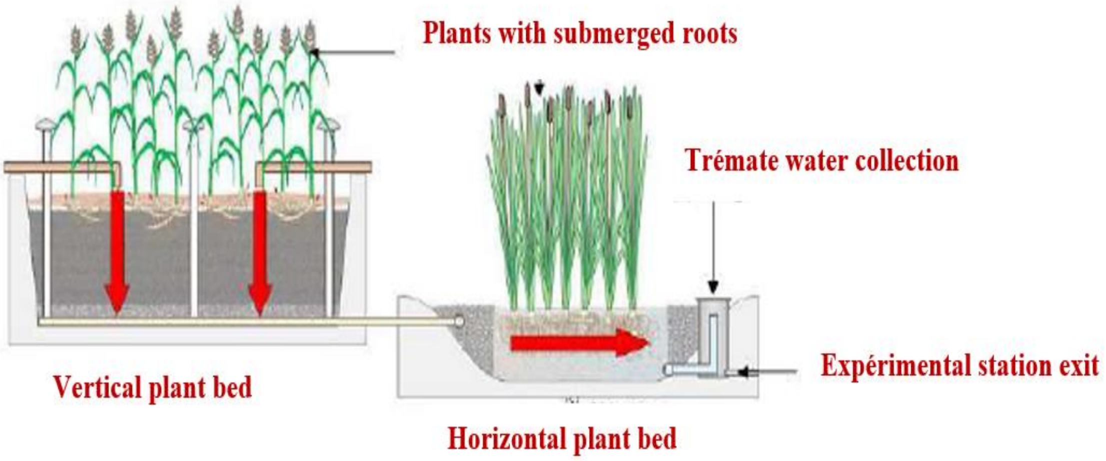

Fig3. A treatment pond with various flow rates

2.3.Methods of sampling and analysis in the laboratory

Samples were collected twice per month (every 15 days) from January to December 2024 at the primary treated water collection warehouse of the classical wastewater treatment plant in Biskra. The collection was performed using 200 L barrels in compliance with the conditions of sample transportation [13][14]. The concentrations of pollutants were determined in accordance with the Algerian standards [15] and standard methods established by the American Public Health Association [16]. For treated water, samples were collected twice per month—one in the middle of each month and another after the residence time in the experimental basins—for both the planted and nonplanted (witness) installations. All laboratory analyses were conducted in the laboratories of the Department of Biology, University of Biskra. The physicochemical parameters, including pH and temperature T (°C), were measured in situ. In the case of raw wastewater, measurements were taken at the warehouse of the Biskra treatment plant after primary treatment, whereas for treated water, measurements were taken at the outlet of the two experimental installations after 15 days of residence via a portable HANNA device (HI 9829). Nitrogenous pollutants were analyzed as follows: ammonium and nitrite concentrations were determined in the laboratory via a spectrophotometric multimeter (WTW Photolab S6), whereas nitrate concentrations were measured via a strip test device (Bandlet test). All the results are expressed in g/L.

3. Quantitative discussion and scientific interpretation of the results

After analyzing the samples in the laboratory, we recorded the results in Table 2 and calculated the purification yield % R for each nitrogen contaminant via Equation (1):

$$R\% = \frac{X_I - X_O}{X_E} \text{ x } 100 \quad \text{.........eq (1)}$$

$\boldsymbol{X_I}$ is the concentration of the pollutant at the inlet of the experimental station. $\boldsymbol{X_O}$ at its outlet.

Table 2: Pollutant concentrations in control and planted ponds before and after 15 days (2024)

		Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
The witness structure	T	X_I	19.4	20.2	22.6	28 .0	29.7	32.0	32.8	33.6	31.4	31.1	29.2	20.8
		X_O	9.30	11.3	15.7	18.0	28.3	30.5	31.2	29.3	27.0	25.2	22.3	11.6
	pH	X_I	7.9	7.8	8.1	7.9	7.9	7.8	7.9	7.8	7.7	7.6	7.2	7.4
		X_O	7.1	7.3	7.5	6.3	7.1	7.3	7.2	7.5	7.2	7.2	7.1	7.1

	NH_4^+	X_I	14.8	17	15.35	12.8	14	18.5	16.7	15.3	12.8	18.5	13.1	12.8
		X_O	6.5	7.5	9.0	5.6	5.9	8.7	5.05	4.8	3.8	3.5	3.9	3.6
	NO_3^-	X_I	54.6	39.2	38.4	28.2	23.2	28.7	32.4	27.2	23.5	34.3	42.9	31.40
		X_O	5.60	8.2	7.60	9.0	12.10	14.1	8.40	5.40	7.6	13.9	8.15	11.20
	NO_2^-	X_I	0.57	0.22	0.39	0.28	0.104	0.13	0.18	0.1	0.11	0.15	0.123	0.170
		X_O	0.08	0.18	0.13	0.10	0.08	0.07	0.08	0.07	0.05	0.04	0.04	0.068
The planted structure	T	X_I	19.4	20.2	22.6	28.0	29.7	32.0	32.8	33.6	31.4	31.1	29.2	20.8
		X_O	10.2	11.4	15.7	17.2	25.3	31.4	31.0	33.5	27.2	25.3	22.4	12.3
	pH	X_I	7.9	7.8	8.1	7.9	7.9	7.8	7.9	7.8	7.7	7.6	7.2	7.4
		X_O	6.8	6.7	6.7	6.6	6.4	6.9	6.8	6.9	6.8	6.6	6.4	6.7
	NH_4^+	X_I	14 .8	17	15.35	12.8	14	18.5	16.7	15.3	12.8	18.5	13.1	12.8
		X_O	4.50	6.30	7.60	3.6	3.8	4.0	3.01	3.9	2.30	2.95	2.15	2.10
	NO_3^-	X_I	54.6	39.2	38.4	28.2	23.2	28.7	32.4	27.2	23.5	34.3	42.9	31.40
		X_O	1.45	7.30	3.20	2.6	5.30	6.3	2.26	2.20	4.40	6.4	5.60	2.30
	NO_2^-	X_I	0.57	0.22	0.39	0.28	0.104	0.13	0.18	0.1	0.11	0.15	0.123	0.170
		X_O	0.05	0.07	0.09	0.08	0.022	0.05	0.09	0.02	0.04	0.03	0.03	0.029

"Table 2 shows the monthly average pollutant concentrations in Biskra’s domestic wastewater before (X_I) and after (X_O) treatment. Wastewater remained 15 days in basins unplanted and Juncus effusus–planted basins during 2024."

The average value of the pollutant concentration ($\bar{C}$) (\overline{C}) was calculated as the sum of the monthly concentrations of the pollutant (Ci) during the study period divided by the total number of months in the year (n=12), as expressed in Equation (2):

$$\bar{C} = \frac{\sum_1^{12} C_I}{12} \qquad \text{eq} \qquad 2$$

We then calculated the purification yield R) for each pollutant by applying relation N^{o}. 1 above), and the results are presented in Table 3 .To prepare Table 3, we denote the percentage of disinfection in uncultivated ponds by R_1 and the disinfection ratios in the cultivated ponds by R_2 .We denote the average annual value of the purification yield by $\bar{R}$.

Table .3 Average purification rates for unplanted basins (R_1), planted basins (R_2), and overall rates ($\bar{R}$) of each pollutant during 2024

R_1		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	$\bar{R}$ %
%	NH_4^+	56.08	55.85	41.36	56.25	57.85	52.97	69.76	68.62	70.31	81.08	70.22	71.87	.6263
	NO_3^-	89.74	79.08	80.20	68.08	47.84	50.87	74.07	80.14	67.65	59.47	81.00	64.33	70.16
	NO_2^-	85.96	18.18	66.66	64.28	23.07	64.15	55.55	30.00	54.54	76.33	67.47	60.00	55.51
R_2	NH_4^+	69.59	62.94	50.48	71.87	72.85	78.37	81.97	74.50	82.03	84.05	83.58	83.59	74.62
%	NO_3^-	97.34	81.37	91.66	90.78	77.15	78.04	93.02	91.91	81.27	81.34	86.94	92.64	86.96
	NO_2^-	89.47	86.18	76.92	71.42	78.84	61.53	50.00	80.00	63.63	80.00	75.60	82.94	74.71

"Table 3 summarizes the monthly and annual average purification rates of pollutants in noncultivated R_1% and planted R_2% basins. The 12-month study highlights $\bar{R}$ %\ overline as the annual mean for each pollutant. "

To ensure the reliability of the graphical analysis and facilitate the representation of the curves, the concentration of nitrogenous pollutants in the wastewater prior to treatment is denoted **X**. Following a 15-day retention period in the ponds, the concentration of these pollutants in the treated effluent is expressed as **X_1**, which corresponds to the concentration measured at the outlet of the unplanted control unit. In parallel, the concentration of nitrogenous pollutants at the outlet of the system cultivated with *Juncus effusus* is denoted **X_2**. This notation provides a clear distinction between the control and planted systems, thereby enabling a more accurate comparison of treatment performance.

To facilitate the discussion and analysis of the results, we adopted two approaches: the first is the quantitative discussion of the results, and the second is the scientific justification of the results for each pollutant monitored in this study.

3.1Annual dynamics of temperature T (°C) in 2024

The variation in temperature during the experimental period at the experimental station is illustrated in **Figure 4**, which presents the temporal fluctuations recorded throughout the duration of the experiments.

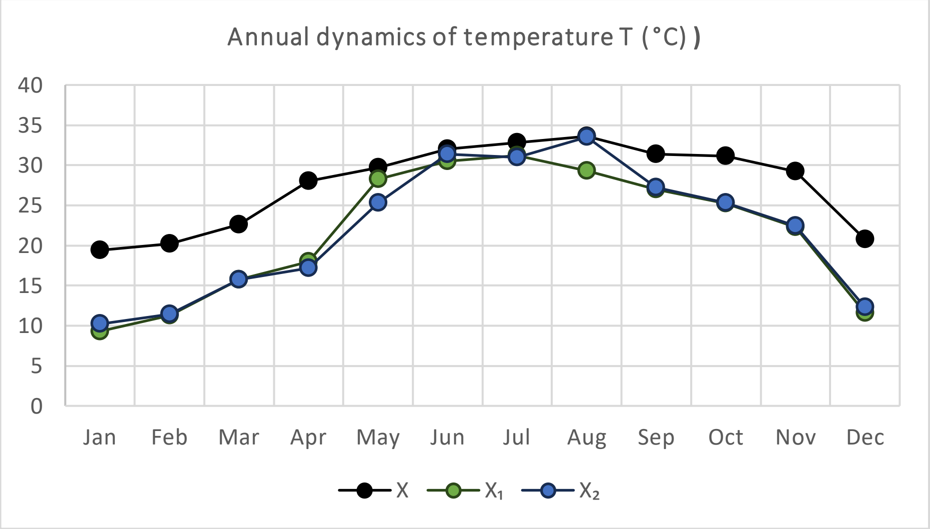

Fig. 4. Annual dynamics of temperature T (°C) in 2024

Quantitative discussion and scientific interpretation:

Fig. 4 shows the variation in temperature for domestic wastewater before treatment (represented by curve **X**), for treated water from the unplanted ponds (represented by curve **X_1**), and for treated water from the ponds planted with the species *Juncus effusus* (represented by curve **X_2**); it appears that temperature varies according to the four seasons of the year. The three curves follow almost the same trend, shifting from lower temperature values in January for curve **X**, which represents the variation in raw wastewater temperature before treatment, where the temperature increased from **19.4°C** recorded in January to **33.6°C** recorded in August, moving from a very cold month to a peak in July. For curves **X_1** and **X_2**, which represent the temperature variation in treated water at the outlets of the unplanted and planted ponds, respectively, the values ranged from a minimum of **9.5°C** and **10.5°C**, respectively, to very close to the maximum values of **31.2°C** and **33.5°C**, respectively, in August, which are also very close. These results are highly consistent with those obtained by [17][18][19][20]**.**

Moreover, the temperatures at the outlets of both experimental systems (**X_1** and **X_2**) are lower than the temperature of the untreated wastewater (**X**). Figure 4 shows that the average temperature values were lower in both the unplanted treatment ponds (control) and the planted ponds than in the raw wastewater. The recorded temperature values ranged from $19.4 < T$ (°C) < 33.6, indicating a noticeable reduction that reflects the influence of the treatment system on the thermal characteristics of the wastewater. The reduction in temperature observed in basins planted and unplanted treatment ponds, compared with the raw wastewater, can be attributed primarily to the decline in bacterial abundance responsible for biodegradation processes, which in turn reduces the intensity of biochemical reactions [21]. However, the outlet water temperatures of both pond systems remain nearly identical. This similarity is explained by the 15-day hydraulic retention period, during which the water is largely insulated from external environmental drivers such as solar radiation and wind dynamics. Under natural conditions, strong wind gusts may increase the water temperature through turbulence-induced exothermic reactions generated by fluid collisions. Since such conditions are absent within the treatment ponds, the resulting temperature profiles exhibit convergence, as reflected in the overlapping patterns of curves (**X_1** and **X_2**). There was no significant difference between the temperature values of the raw wastewater and those of the ponds planted with the studied plant or the unplanted control ponds. These temperature variations are attributed primarily to climatic factors that regulate the increase and decrease in temperature, as clearly illustrated in Figure 4. Such variations are referred to as thermal exchange with the external environment. The average temperature of the treated water in the control ponds (**X_1**) and in the planted ponds (**X_2**) remained below 30°C, which is the standard threshold adopted in Algeria for agricultural irrigation water (Official Journal of the People's Democratic Republic of Algeria. **2006**, Executive Decree N°. 93--160 of July 10**)**. Moreover, this average temperature falls within the optimal range for supporting biological processes in plants. The values obtained during the 2024 study period regarding temperature variations are comparable to those reported by Dovonou [22] in their research conducted in southern Benin and by Manaia [42]**.**

3.2. Annual dynamics of acidic pH) in 2024

Fig 5. Variations in the acidity (pH) values of the wastewater before treatment and of the treated effluent discharged from the unplanted ponds compared with the corresponding values of the treated water from the planted basins
throughout the research year 2024. The figure highlights the differences in acidity trends among the three systems, thereby reflecting the effects of macrophyte-based treatment on the stabilization and regulation of pH levels in wastewater**.**

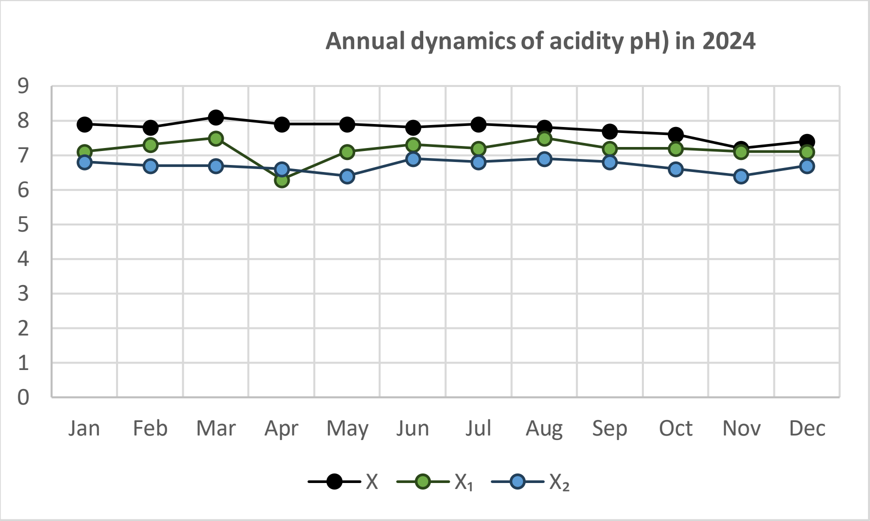

Fig 5. Annual dynamics of acidic pH in 2024

Quantitative discussion and scientific interpretation:

Monitoring the changes in pH levels shown in Fig. 5 revealed three distinct levels. The first level corresponds to the values of raw wastewater before treatment (Curve X), where the pH ranged from a minimum of 7.2 in November to a maximum of 7.9 in January, April, and May, July. This fluctuation suggests that the acidic components in household wastewater remain relatively stable throughout the year, whereas the observed increase in pH is largely associated with the intensive use of alkaline cleaning agents and detergents. The average pH recorded during the 2024 study period for this untreated water was 8.4, confirming its alkaline character. These basic conditions indicate a strong anthropogenic influence related to domestic practices, particularly the frequent use of detergents with alkaline properties. These results are consistent with the findings of [15][19][23] and are comparable to the wastewater characteristics reported in Ouagadougou, Burkina Faso [23]. The second level, represented by curves X_1 and X_2, shows a decrease in acidity values compared with curve X, indicating a modification of the wastewater characteristics after treatment. In the unplanted ponds (X_1), the pH values ranged between 6.3 in April and 7.5 in March and August, with an average of 7.15. This suggests that the gravel and sand components contributed to the adsorption of basic species (OH^-), causing the initially basic raw wastewater to become neutral. In the planted ponds (X_2), the pH values ranged from 6.4 (May and November) to 6.9 (July and August), with an average of 6.69. This finding indicates that the treated water became slightly acidic due to the activity of the plant roots and associated rhizospheric bacteria, which increased the absorption of basic compounds. These findings are consistent with the results of [20][24] but differ from those reported by Hammadi

[19]. These results are quite similar to the results obtained[22] in Benin. Fig. 5 (curve X) shows that the pH values were slightly above the equilibrium value of 7, ranging between 7.4 and 8.1, with an annual average of 8.4 in 2024. This alkalinity is attributed mainly to the activity of plankton, algae, and anaerobic bacteria, as well as the decomposition of bicarbonate acids in groundwater into water and carbon dioxide [25] Reaction 1

HCO_3-H + OH-HCO_2 $\rightarrow$ 2 H_2O + 2 CO_2 Rea 1

Previous studies have also reported an increase in pH during summer due to these biological processes [26]. Our results confirmed this trend, with the highest pH values (7.9, 7.8, 7.9, and 7.8) recorded in May, June, July, and August, respectively, coinciding with intensified photosynthetic and biochemical activities. A clear decrease in pH was observed in both systems (planted with Juncus *effusus* and unplanted). In the unplanted system (curve X_1), the values ranged from 6.3 in April to 7.5 in March and August, with an annual average of 7.15 in 2024, indicating a shift toward neutrality. In the planted ponds, the values ranged from 6.4 in May and November to 6.9 in August, with an average of 6.69, suggesting a tendency toward slight acidification. This decrease is attributed mainly to bacterial metabolism, particularly the activity of autotrophic nitrifiers such as *Nitrosomonas* and *Nitrosococcus* (Reaction 2), which oxidize ammonium (NH_4^+) to nitrite (NO_2^-) while releasing protons (H^+). The release of protons explains the acidification, while the endothermic nature of this reaction makes it highly dependent on temperature and oxygen availability.

$NH_4^+ + 3/2\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O$ Rea……2

3.3. **Annual dynamics of ammonium concentration NH_4^+ (mg/L) in 2024**

The variation in ammonium concentration throughout the duration of the experiments conducted at the experimental station is illustrated in Fig. 6. Two distinct trends can be observed, reflecting the temporal dynamics of ammonium levels during the study period.

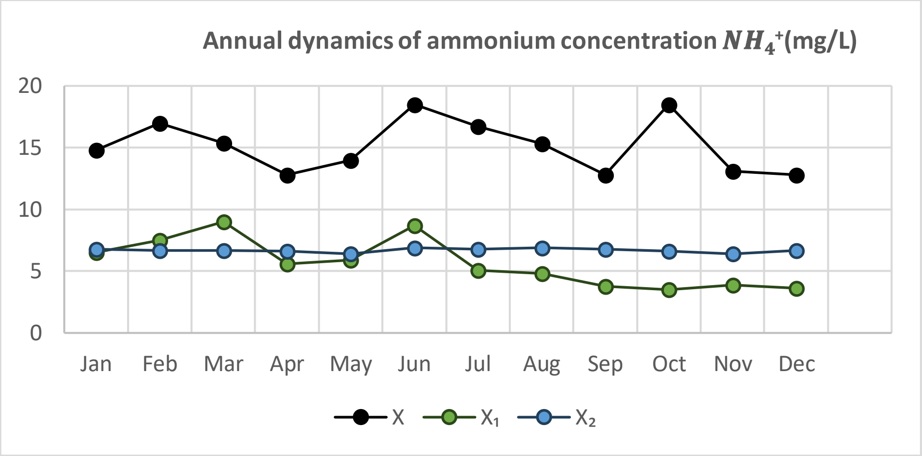

Fig 6. Annual dynamics of ammonium **concentration NH_4^+(mg/L)** in 2024

Quantitative discussion and scientific interpretation:

The concentration of ammonium (NH_4^+) varied significantly between raw and treated water. In the raw wastewater (curve X, Fig. 6), the minimum ammonium concentration was 12.8 mg/L, recorded in April and December, whereas the maximum concentration was observed in June, with an overall average of 15.15 mg/L during the study period. In the treated water from the unplanted ponds (control), represented by the curve X_1 in Fig. 6, the ammonium concentrations ranged from a minimum of 3.5 mg/L in October to a maximum of 8.7 mg/L in June, with an average value recorded at the outlet throughout the year 2024. With respect to the planted basins with the species *Juncus* effususus, the ammonium concentrations varied between a minimum of 2.10 mg/L observed in December and a maximum of 7.60 mg/L noted in March. The average value recorded at the outlet, after a retention time of 15 days in the treatment ponds, is represented by the curve X_2in Fig. 6. Overall, these values are consistent with those reported by Tama[28]. According to the results presented in Table 3, the purification yield of ammonium in the nonplanted ponds (R1%R_1\%) showed substantial temporal variability, ranging from a minimum of **41.36%** in March to a maximum of **81.08%** in October, with an annual average of R¯ %=62.63\overline R \% = 62.63. In comparison, the ponds planted with *Juncus effususus* exhibited consistently greater purification performance, with R_2% values varying between **50.48%** (March) and **83.59%** (December) and an annual mean of R¯ %=74.62\overline R\% = 74.62. These findings clearly demonstrate the positive influence of vegetation on ammonium removal efficiency. Furthermore, the purification yields obtained in the planted system were greater than those reported by Bendida and Tama [20] [28] lower than those recorded by Petemanagnan Ouattara [27] and closely aligned with the values reported by Hammadi

[19]. These outcomes highlight the potential of macrophyte-assisted treatment systems to increase ammonium removal through synergistic interactions between plants and microbial communities. The decrease in temperature values within the ponds designated for the treatment of raw wastewater, compared with their values outside the ponds before treatment, plays a significant role and strongly influences the reduction in ammonium concentrations [29]. This can be attributed to the storage of wastewater in the ponds, which makes it less dynamic and reduces the chances of collisions between water molecules. As a result, the reactive mobility becomes less effective in dissipating heat, thereby creating a favorable environment for bacterial functions involved in the removal of this pollutant. Moreover, the removal concentrations were greater during the months characterized by elevated temperatures, as evidenced by the values presented in Table 2. In the study area, temperatures remain high from April to October, which leads to increased volatilization of ammonia [30] . In addition, the vertical irrigation of ponds contributes to variations in temperature, which in turn generates thermal fluctuations that significantly promote algal growth. This algal dominance affects the photosynthetic process and hydrodynamics of water flow in ponds while also playing a role in nutrient uptake and recycling [31][32][40]. The reduction in ammonium concentration (N-NH_4^+) in both planted and unplanted ponds occurs through two successive oxidation processes, in which ammonium is first converted into nitrite (NO_2^-) and subsequently into nitrate (NO_3^-) under aerobic conditions. Autotrophic bacteria play a crucial role in these processes by colonizing the root zone, where they increase the availability of dissolved oxygen in the water, particularly during photosynthesis. The overall nitrification process, whereby ammonium is oxidized to nitrite and then to nitrate, can be summarized by the following two reactions:

$$4\,NH_4^{+} + 7\,O_2 \text{ (Nitrosomonas)} \rightarrow 4\,NO_2^{-} + 6\,H_2O + 4\,H^{+} \qquad \text{Rea 3}$$

$$NH_4^{+} + \frac{183}{100}O_2 + \frac{198}{100}HCO_3^{-} \rightarrow \frac{21}{1000}C_5\,H_7NO_2 + \frac{104}{100}H_2O + \frac{98}{100}NO_3^{-} + \frac{188}{100}H_2CO_3 \qquad \text{Rea 4}$$

3.4. **Annual dynamics of nitrate concentration NO_3^-(mg/L) in 2024**

Fig. 7 shows the variations in nitrate concentrations in wastewater before treatment and at the outlet of the unplanted ponds (control) after a specified retention time of 15 days. Furthermore, it highlights the temporal changes in nitrate concentrations during the 2024 study period in the treated water exiting the ponds planted with the species Juncus effusus. The figure clearly shows the comparative differences between the unplanted control system and the planted system, emphasizing the role of vegetation in enhancing nitrate removal efficiency over time.

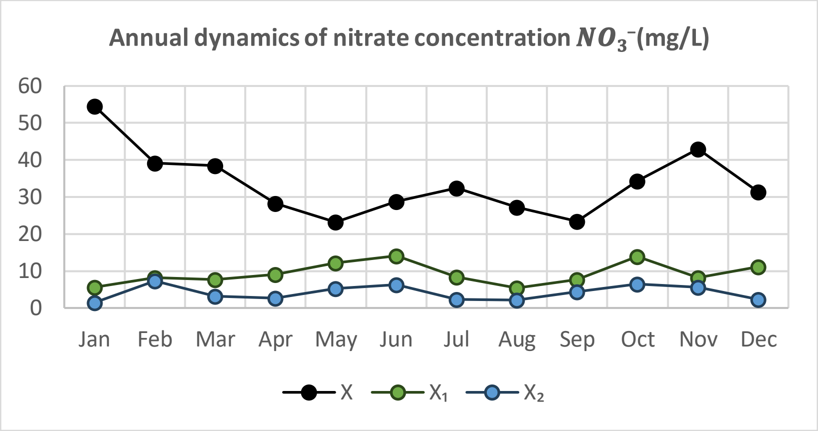

Fig 7. Annual dynamics of nitrate concentration NO_3^-(mg/L) in 2024

Quantitative discussion and scientific interpretation:

The nitrate concentration in the raw water used for irrigating the planted basins ranged from 23.2 mg/L in May to 54.6 mg/L in January, with an annual average of 33.6 mg/L. At the outlets of the planted basins, the values varied between 1.45 mg/L in January and 7.30 mg/L in February, with a mean of 4.10 mg/L, remaining well below the guideline value set by the FAO (< 50 mg/L) and the WHO. The annual purification efficiency reached 87.79%, exceeding that reported by Hammadi [19] and far higher than the 36.13% achieved by Bensalman [33]. The highest removal efficiency (97.34%) was recorded in January, whereas the lowest (77.15%) occurred in May. Overall, the planted basins achieved superior purification efficiency (87.79%) compared with the unplanted control basins (70.16%). The reduction of nitrate (NO_3^-) in domestic wastewater treated with plants occurs primarily through heterotrophic denitrification under oxygen-limited conditions, which uses organic carbon as an electron donor [34]]. In this process, nitrate reacts with the organic compound CH_2OHCH_3 in the presence of hydrogen ions H^+, producing biomass $C_5H_7NO_2$, carbon dioxide CO_2 (partly utilized in photosynthesis), water H_2O associated with transpiration, and molecular nitrogen (N_2). This reduction involves the transformation of nitrogen from an oxidation state of +5)in nitrate) to 0)in dinitrogen gas). Reaction 5 summarizes this mechanism, highlighting the integrative biochemical relationship between plants and bacteria, which reflects the principles of green chemistry.

$$\mathbf{97}NO_3^- + 50\ CH_2OHCH_3 + \mathbf{97}\ H^+ \rightarrow \mathbf{5}\ C_5H_7NO_2 + \mathbf{75}\ CO_2 + \mathbf{181}\ H_2O + \mathbf{46}\ N_2 \qquad \text{Re: } 5$$

Organics mater Biomass

This biochemical reaction can also be expressed in a concise form, clearly illustrating the reduction pathway of nitrogen from nitrate ions to atmospheric nitrogen (Reaction 6).

$$NO_3^- + 6H^+ + 5e^- \rightarrow \frac{1}{2} N_2 + 3 H_2O \qquad \text{Re: 6}$$

3.5.**Annual dynamics of nitrite concentration NO_2^-(mg/L) in 2024**

The variations in nitrite concentration throughout the study period of 2024 in the domestic wastewater of the city of Biskra were monitored and analyzed before and after treatment, both in the noncultivated ponds (control) and in the ponds cultivated with the study plant Juncus effusus, as illustrated in Figure 8.

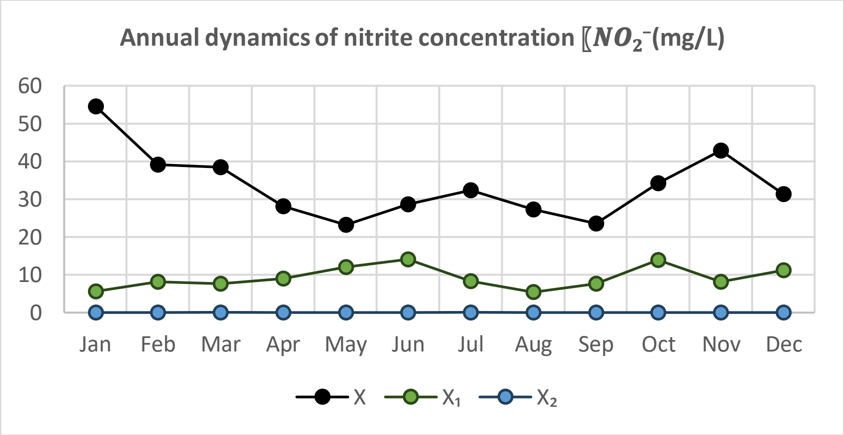

Fig 8. Annual dynamics of **nitrite concentration NO_2^-(mg/L)** in 2024

Quantitative discussion and scientific interpretation:

The nitrite concentration in raw domestic wastewater before treatment ranged from 0.10 mg/L in August to 0.57 mg/L in January, with an annual mean of 0.21 mg/L at the inlet of the unplanted ponds in 2024. After a 15-day retention period, the nitrite concentration in the unplanted ponds decreased to values between 0.04 mg/L (October–November) and 0.18 mg/L (February), with an annual mean of 0.08 mg/L, corresponding to a purification efficiency of 61.90%. In the ponds planted with *Juncus effusus*, nitrite concentrations varied between 0.020 mg/L (in August) and 0.05 mg/L after 15 days of retention, yielding an average purification efficiency of 76.19% during 2024. This value is lower than that reported by Hammadi [19] (83.06%) but higher than that obtained by Middlebrooks [35]52.4%. Overall, the nitrite removal efficiency during the study period ranged from 51.55% to 89.47%, which reflects highly satisfactory treatment performance. In fact, the elimination of nitrite proceeds via two distinct reactive pathways. The first pathway involves a stepwise chemical process in which nitrite is initially oxidized to nitrate, followed by the subsequent reduction of nitrate to atmospheric nitrogen, leading to the release of diatomic nitrogen gas (N_2) and the formation of water, as represented in Reaction 7. The second pathway consists of the direct reduction of nitrogen, where it undergoes a redox transformation from an oxidation state of (+5) to a gaseous form with an oxidation state of (0), concurrently producing water molecules, as depicted in Reaction 8 [30][41][43].

$NO_2^- + \frac{1}{2}O_2 \rightarrow \quad NO_3^- + 6H^+ + 5e^- \rightarrow \frac{1}{2} N_2 + 3\,H_2O$ Re ……7

$NO_2^- + 4\,H^+ + 3\,e^- \rightarrow \frac{1}{2} N_2 + 2\,H_2O$ Re …. 8

4. Conclusion

Through their stems, leaves, and structural surfaces, aquatic plants provide favorable habitats for microbial colonization and growth [36][37][38]. Biofilms form on plant tissues that host bacteria and dense algal communities, whereas roots in moist soils further enhance these microhabitats [39]. These interactions play crucial roles in wastewater treatment by promoting the removal of nitrogenous pollutants through bacterial and algal activities. On the basis of these ecological interactions, a pilot-scale station was established in Biskra to treat domestic wastewater with primary treated influent. The system comprised two configurations: an unplanted setup and a planted setup with *Juncus effususus*. Each included three basins (150 L each), with a substrate of 10 cm sand over 50 cm gravel, operated under vertical and horizontal flow regimes. The hydraulic retention time (HRT) was set at 10 days in the first two basins and 5 days in the third basin. Irrigation was conducted twice per month, and the effluent was analyzed every 15 days. The study pursued scientific, environmental, and social objectives. Specifically, this study sought to analyze the eco-biological mechanisms governing plant–microbe interactions in nitrogen removal and to assess plant diversity in enhancing nitrification–denitrification processes. Environmentally, it aims to reduce nitrogen pollution, improve water quality, and promote low-carbon treatment alternatives. Socially, it aspires to protect public health, raise environmental awareness, and generate local employment. The results revealed substantial nitrogen removal. The ammonium (NH_4^+) concentration ranged between 2.10 and 7.60 mg/L, with a mean value of 3.62 mg/L, corresponding to a removal efficiency of 74.62%. The mean nitrate outlet concentration was 4.10 mg/L, with an 87.79% removal efficiency. The average nitrite concentration was 0.05 mg/L, with a 76.19% removal efficiency. The overall nitrogen removal efficiency reached 79.33%. The hybrid vertical–horizontal flow constructed wetland with *Juncus effususus* met Algerian standards for agricultural reuse, offering ecological adaptability, low cost, and high sustainability for wastewater treatment in arid and semiarid regions.

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